Chapter 8

General summary, discussion and conclusion

This thesis investigated the effects of several specific dietary components on some of the risk factors for CHD. The different diets were a high lycopene diet with monounsaturated fat (light olive oil) or high carbohydrate; and a diet containing chilli versus a bland diet. Following is a précis of the findings from the two research projects.

8.1 Tomato olive oil combination: A step towards reducing CHD risk?

There were a number of reasons for choosing to study a tomato-light olive oil combination. While lycopene, a lipophilic carotenoid antioxidant, present in tomatoes is thought to be relevant in reducing the risk for CHD, controversy exists as to whether the absorption and hence serum concentrations of lycopene is altered if tomatoes are consumed in the presence of high or low fat diets. Secondly, after 60 years of research there remains an ongoing debate whether monounsaturated fatty acids or carbohydrates are the better substitutes for saturated fatty acids to reduce the risk factors for CHD. Finally, although extra virgin olive oil is considered beneficial for health due to the presence of MUFA rich oleic acid and polyphenol antioxidants, it has a strong flavour and is expensive. Light olive oil is milder in taste and less expensive. However, it needs to be determined whether the lycopene-light olive oil combination raises serum lycopene and is preferable to a lycopene carbohydrate combination for its effects on some of the risk factors for CHD.

The study undertaken included two dietary periods of ten days each, both rich in lycopene, but one was high in monounsaturated fat (33 to 36% of energy as fat), while the other was high in carbohydrates (15 to 16% of energy as fat). The study was based on weight maintenance, randomised crossover design. The intervention diets were iso-energetic to the participant's usual diet and were matched for energy, protein, lycopene and fibre content. The sources of lycopene in the two diets were tomato soup and tomato paste with light olive oil as the source of fat. The effects on serum lycopene concentrations, lipids and lipoproteins and serum lipid peroxidation were investigated.

The results of this study (Chapter 2) confirmed our earlier data (342) and indicates that after ten days of 16% or 35% of energy from fat in the diet, accompanied by high amounts of lycopene, increases the serum lycopene concentrations to a similar level. Both diets led to a lower serum total and LDL cholesterol to similar levels. Although HDL cholesterol was higher, triglycerides and LDL to HDL ratio were lower on the MUFA rich diet compared to the carbohydrate rich diet (as has been shown by a number of earlier studies), these results from present investigation should be treated with caution because the diets were of short durations (10 days each).

Contrary to the previous study showing an effect of MUFA on susceptibility of isolated LDL to oxidation (270), there was no significant effect of light olive oil on whole serum lipid oxidation. In addition to the different test mediums (isolated LDL versus serum), other reasons for this discrepancy in results could be different research designs and/or the source of MUFA. While the dietary periods for the earlier research were 14-16 days and the source of MUFA was oleic acid and vitamin E rich sunflower oil, the dietary periods for the present investigation were ten days and the

source of MUFA was light olive oil, which has considerably lower amounts of vitamin E.

In conclusion, the results of the tomato olive oil study suggest that 10 days of high lycopene high monounsaturated fat and high lycopene carbohydrate rich diets present similar increases in serum lycopene concentrations.

8.2 Chilli: hype or hope? A bit(e) of both

Although chilli has been used in folk medicine for the treatment of diseases and disorders including whooping cough, diphtheria, diabetic neuropathy and in modern medicine (capsaicin-the active ingredient of chilli) for the management of pain, little information is available about its effect on risk factors for CHD, especially in humans. The little information that is available suggests that capsaicin has antioxidative properties and reduces the susceptibility of LDL to oxidation *in vitro*. Some animal data reports a potential beneficial effect of chilli in reducing serum total and LDL cholesterol, high concentrations of which are among the major risk factors for CHD. Some research also suggests that topically used capsaicin has vasodilatory effects, while other research reports that ingestion of chilli with a meal leads to increased metabolic rate. This particular finding (increase in metabolic rate) brought the chillies to the public interest and today most people perceive that chilli may help in reducing body weight. It has been suggested that this increased metabolic rate is the result of increased SNS activity, as serum catecholamine concentrations have been found to be increased after the ingestion of meals containing chilli. However to our knowledge no data is available to show that regular chilli intake leads to weight reduction. The data that showed an increase in the metabolic rate was collected from small groups (n = 8 to 13) of young lean healthy individuals (mean age 20 to 25 years and mean BMI of 20 to 24 kg/m^2; mainly marathon runners), whose metabolism may be different to that of the sedentary or moderately active general public. Secondly, the findings of increased metabolic rate were the result of single chilli meals trials and it is not known whether regular intake of chilli demonstrates similar or different results. Finally, dried chilli powder, the form in which chilli was provided in the previous studies, is easier to add/mix in curries, pasta and rice, but may be difficult to

incorporate in usual Australian cuisine which is often primarily based around bread (sandwiches), roast/ grilled meat and vegetables.

Our human dietary intervention study aimed to investigate the metabolic and vascular effects of regular chilli consumption (for four weeks), and compare these to the effects from a bland diet for four weeks, in 36 individuals from the general public with a mean age of 46 years (range 22 years to 70 years) and BMI of 26kg/m^2 (n = 12 with BMI < 25 kg/m^2; n = 17 with BMI of 25 to 29.9 kg/m^2; and n = 7 with BMI ≥ 30 kg/m^2). This particular participant group was chosen to provide the opportunity to examine the effects in a wide range of people with respect to age and BMI. Additionally, as none of the participants were taking any prescribed medication any results observed could be considered to be due to the action of chilli and not due to the interaction between chilli and the drugs. Although a small number of participants (six) were taking vitamin supplements before and during the intervention studies, this intake was kept consistent between the two dietary periods. In addition, these participants taking vitamin/antioxidant supplements were excluded from the oxidation study (Chapter 4). The study was also designed to investigate the effects of two chilli-supplemented meals (one consumed at the end of the bland diet and the other consumed at the end of the chilli diet) on a range of metabolic and vascular parameters, and compare the results to those of a bland meal (consumed after three weeks of the bland diet). This methodology was decided upon from the information that habitual diets may affect the activity and responsiveness of receptors involved in regulation and transport of nutrients. The measured parameters included body weight, fat and lean mass, serum lipids and lipoproteins, serum lipid oxidation, glucose, insulin, metabolic rate, heart rate, peripheral and aortic blood pressure, augmentation

index (AIx; an indicator of arterial stiffness) and subendocardial viability ratio (SEVR; a measure of myocardial perfusion).

The study was conducted using a randomized crossover study design, with a weight maintenance regime. As more than 80% of the participants were infrequent or naïve consumers of chilli, it was difficult for them to eat the whole amount of chilli blend (30g/day; 55% cayenne pepper, sugar, water and food acid) in one meal. Chilli blend was used in the study rather than dried chilli powder (as has been used in earlier research) for the following reasons. In addition to adding the chilli in soups, pasta and rice, it was possible to consume part of the daily quota of chilli as spread on bread (most common ingredient in Australian's lunch) and then the other half mixed with yoghurt as an accompaniment to roast meat/ vegetables in the evening meal. This helped in spreading the full day's chilli intake in 2-3 different meals. The particular dose of chilli was decided upon after palatability tests with some colleagues (infrequent consumers of chilli), in which 32-35g of the product ('Freshly Chopped Chilli' MasterFoods® Australia) was found to be most acceptable and feasible. To make things easier for the investigators and participants, we could have used capsaicin supplements instead of the chilli blend; however the aim of our investigation was to examine the effect of a food that provides taste and palatability to the diet and not a supplement taken as a medication.

During the two dietary periods (in week three of each diet), the endothelium-independent and endothelium-dependent vascular responses of fifteen participants were also assessed using glyceryl trinitrate (GTN) and salbutamol, respectively. The baseline AIx (augmentation index, an indicator of arterial stiffness) at three weeks (on the day of the drug test) on the chilli diet was significantly lower than on the

bland diet, however the overall effects of the two drugs were not significantly different between the two diets (Chapter 3). This suggests that regular chilli consumption does not augment or encumber the acute effects of the two major vasodilatory drugs in healthy individuals. Further research is required to test if different results are observed in people with endothelial dysfunction at baseline.

Four weeks of iso-energetic chilli and bland diets (with a weight maintenance regime) did not show any significant difference in the body fat and lean mass, serum lipids, lipoproteins, glucose, insulin, blood pressure, heart rate, AIx or SEVR. A significantly lower (by 2.5 beats per min) resting heart rate was observed after the chilli diet compared to the bland diet in men, but not in women. This result may have significance, for elevated heart rate (especially in Caucasian men) is an independent risk factor of CVD and total mortality (459, 503, 504). The basal metabolic rate at the end of the two dietary periods was also similar and there was no significant difference for the fat and carbohydrate oxidation (Chapter 3). During our data collection, Lejuene et al. (2003) published similar findings (for lipids, glucose, insulin and metabolic rate) for their study investigating the effect of 135mg/day of capsaicin supplement (for nine weeks) in overweight individuals (390).

In the whole group, rate of copper-induced serum lipoprotein oxidation was found to be significantly lower at the end of the chilli diet compared to the bland diet (Chapter 4). In women but not in men, the lag phase before oxidation was longer with the chilli diet (by 9.6 min). This increased lag phase may have been the result of higher chilli/capsaicin intake per kg body weight in women compared to men. Although this assumption was supported with the *in vitro* study (Chapter 5), where serum from healthy individuals (not on any experimental diets) was incubated with different

concentrations of capsaicin, dihydrocapsaicin or the active ingredient of turmeric – curcumin and induced to copper oxidation, we are unable to confirm the findings. Analyses of serum capsaicin levels may have added some support to my assumption, but we were unable to perform that analysis. The *in vitro* study showed that serum with increasing concentrations of capsaicin, dihydrocapsaicin or curcumin, resisted copper-induced oxidation for longer periods of time. These results are of significance if this susceptibility occurs *in vivo,* as oxidised LDL may play a significant role in the development of atherosclerosis.

I acknowledge that the sub-group analyses performed in Chapters 3, 4 and 6 should be viewed with caution as originally the study was not designed to separate the data between men and women or between participants in different BMI ranges and this may impact on the power of the analyses performed.

As reported earlier, in addition to the four week regular chilli consumption study, the participants also took part in three meal tolerance tests, where postprandial metabolic and vascular responses (up to 2hrs) *after* the bland and the chilli-containing meals were measured and compared. The three meals included: a bland meal after the bland diet (BAB; bland diet after a bland diet); a meal containing 30g chilli blend at the end of the bland dietary period (CAB; chilli meal after the bland diet); and a meal containing 30g chilli blend at the end of the chilli dietary period (CAC; chilli meal after the chilli diet). The meals were matched for energy, carbohydrate, fat, protein and fibre content.

Results for the meal tests (Chapter 6) showed a significantly lower (47%) maximum increase in insulin as well as overall postprandial insulin (39%) after the CAC meal

than the BAB meal, suggesting a lower postprandial requirement of insulin with regular consumption of chilli. Although the plasma glucose concentrations after the chilli meals were 10% lower than the bland meals, this result was not statistically different between the three meals. The reduced/improved postprandial serum insulin response was more pronounced in participants with increased BMI ($\geq$26.3kg/m^2). One of the probable reasons for this improved serum insulin profile in individuals was a 6% reduction in insulin secretion and about 30% increase in hepatic clearance of insulin after the CAC meal compared to the BAB meal. As the study was not designed to directly measure the insulin secretion and clearance, we are unable to confirm this assumption.

Animal studies have reported that the liver is a major deposition site for capsaicin. It is possible that regular intake of chilli improved the insulin receptors activity/number in the liver which increased the heaptic insulin clearance, and possibly led to a reduced SNS activity and hence lower energy expenditure especially in people with higher BMI, after the chilli meals compared to the bland meal. The same reasons may explain the higher SEVR (an indicator of myocardial perfusion pressure) in participants with increased BMI on the CAC meal compared to the BAB meal (Chapter 7). The results for the CAB meal, for all the above mentioned parameters, were in between the results for the CAC and the BAB meal. This suggests that occasional chilli consumption may help in ameliorating postprandial hyperinsulinemia and increasing myocardial perfusion but not to the same extent as the regular chilli consumption.

This study failed to reproduce the results (especially between BAB and the CAB meals) for energy expenditure observed in previous three studies (365, 367, 408).

These previous studies (conducted in lean healthy males and females with BMI of less than 24 kg/m^2) reported higher energy expenditure after the consumption of a meal containing chilli compared to a bland meal. However other studies (409, 410) have also not shown an effect. Although the precise reason for the discrepancy between the results is unclear, it may be the due to the differences in study design, activity pattern or the type of chilli used. While the present study controlled for the background diets and activity pattern of the subjects for four weeks, previous studies that reported an increased EE controlled the diets for a maximum of 48 hours prior to the testing. Although the subject group in the present investigation was primarily Caucasian (33 Caucasian, 2 Indian and 1 Chinese), similar to that in the previous studies, it was mainly from sedentary or moderately active groups compared to the marathon runners in earlier research, and their metabolism may be different. It is expected that the insulin response of marathon runners with higher lean body mass could be more efficient than the sedentary/moderately active group in the present investigation. It may be possible that chilli influences both insulin response and SNS activity directly, however in people with higher fat mass, the effect of chilli on hepatic insulin clearance overrides the direct SNS effect and the lower energy expenditure results from the reduced SNS activity as a response to improved hepatic insulin clearance and hepatic vasodilation.

The results observed in the present chilli research are surprising, unexpected and exciting and may be relevant for the prevention/treatment hyperinsulinemia and metabolic syndrome – the major consequences of obesity. Some of the dietary treatments for reducing postprandial hyperinsulinemia and obesity include increased intake of dietary fibre and low glycaemic index foods. Earlier single meal studies have suggested that chilli produces a perception of oiliness in food and reduces the

desire to eat more (365, 366). Combining the two would probably mean a low energy density, low GI, but increased (and possibly improved) taste and flavour. Chilli may also be used as a substitute to salt and a means to reduce sodium intake. As the findings from this research suggest a beneficial role of chilli in people with increased BMI, it would be interesting to further this work in people diagnosed with insulin resistance and/or Type 2 diabetes.

Although this research study measured a range of metabolic and vascular parameters, other measurements such as the analyses of the number and capacity of insulin receptors, heart rate variability (a measure of SNS activity), nitric oxide (a measure of vasodilation) and glucagon-like peptide 1 (GLP-1; a measure of gastric emptying) would have further added information to some of the research findings and speculations. It would also be interesting to analyse the serum capsaicin concentrations and the time (*after* the meal) at which the serum capsaicin peak to further our understanding concerning the relationship between the chilli/capsaicin intake and the insulin response (We are currently in process of setting up an HPLC method to study this).

Confirmation of the data from Matsumoto et al. (411) on chilli and SNS activity and our results and postulations (Chapter's 3-7) may further ignite the debate between obesity with insulin sensitivity and reduced SNS activity; and obesity/leanness with insulin resistance and SNS hyperactivity. While hyperinsulinemia (a risk factor of CVD) is a product of increased insulin secretion and reduced insulin clearance in (mainly) obese and insulin resistant people, increased SNS activity (possibly a by-product of hyperinsulinemia) is also an independent risk factor for CVD. Further research is also justified to explore the effects of habitual intake of chilli over longer

periods to examine whether the results observed in the present investigation are maintained or are lost/reversed over long periods. It would also be interesting to study whether habitual consumption of chilli in an *ad libitum* diet causes an increase in body weight due to reduced SNS activity/EE, or whether it may actually help in reducing body weight as a result of increased insulin sensitivity, efficient uptake of glucose by the cells and possibly changes in other appetite and weight controlling hormones (including leptin and ghrelin). Research is also required to establish the minimum amount of chilli to be consumed to show the beneficial effects. Although all participants in the present investigation, expect one, were able to tolerate the prescribed amount of chilli, most of them (regular as well as occasional users) suggested that higher or even the same chilli intake would not be possible over longer periods of time. One of the reasons for this remark was that because other spices were forbidden chilli overpowered the taste of all food. Other spices such as turmeric, cinnamon and fenugreek have also been reported to have beneficial effects on health and it would be interesting to test whether the combination of these spices with chilli (as often used in curries) can enhance the favourable health effects as well as increasing palatability.

In addition to demonstrating a potential beneficial role of regular chilli consumption, this study further opens an area in nutrition (and possibly pharmacological) research to understand the mechanisms linking chilli and other spice intake to insulin resistance, obesity and other related disorders.

Conclusion

The results from this thesis research are original and extend our knowledge and understanding about the effects of diets rich in certain foods on some CHD risk factors. Use of tomatoes and light olive oil may help increase serum lycopene concentrations; reduce serum total and LDL cholesterol. Addition of chilli may help reduce the susceptibility of serum lipids to oxidation, ameliorate postprandial hyperinsulinemia, especially in people with increased BMI. The two studies together indicate an opportunity to prepare palatable and practicable diets containing tomatoes, olive oil and chilli that may aid in the prevention of CHD.